Novel Features Revealed by the Three-Dimensional Structure of S100A12 Solved by MAD Phasing

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Proteins of the S100 family mediate a variety of cellular responses to increased Ca^{2+} levels by binding Ca^{2+} and undergoing a conformational change, which allow them to bind to and activate specific target proteins. S100A12 binds drugs, which are known to inhibit IgE-mediated degranulation of mast cells and basophiles and is thus believed to play a role in the allergic response. Like other S100 proteins, S100A12 consists of tandem helix-loop-helix structures; each loop is an EF hand motif, which binds a single Ca^{2+} ion. Crystals of S100A12 in space group $P4_12_12$ were grown in the presence of the lanthanide Tb^{3+}. MAD data was collected to a resolution of 2.5 Å on a frozen crystal at NSLS on beamline X8C. The structure was solved and refined using CNS. Each protein molecule bound 2 Tb^{3+} which gave rise to an enormous anomalous signal. The Tb^{3+} ions were readily located in the anomalous Patterson map. The initial electron density map, calculated with MAD phases to 2.7 Å, was of excellent quality. To the best of our knowledge, this represents the first time that the anomalous signal of Tb^{3+} has been used to solve a protein structure by MAD phasing. Subsequent model building and refinement has led to a current model with an R of 0.27 and R_{free} of 0.29. The same overall structure as seen in other S100 proteins was found. Substantial differences were observed in the loop connecting the two helix-loop-helix motifs and particularly in the C-terminal region of the polypeptide. The specificity of S100A12 for its particular target proteins and for the anti-allergic drugs likely resides in these areas. Interestingly, one of the Tb^{3+} is bound to the C-terminal EF hand, while the N-terminal EF hand is unoccupied. In other S100 proteins, the C-terminal Ca^{2+}-binding site is known to have a significantly higher affinity for Ca^{2+}. The unoccupied EF hand has a very different conformation from the occupied site. The second Tb^{3+} is bound to an unusual site near the N-terminus of the protein which has a distorted octahedral geometry. In spite of their mutually repulsive positive charge, the two Tb^{3+} ions are located only 5 Å from each other.

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